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Comparative Study of Bio-Cellulose from *Acetobacter Xylinum* 0416 and Commercial Hard Gelatine Capsule

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Abstract

Drug delivery technology using natural polymers resources based drug is more preferable by pharmaceutical industry due to their readily available, inexpensive, biocompatible, and potentially degradable. A biopolymer known as bacterial cellulose (BC) is one of the potential of drug delivery materials because it has the ability to produce higher purity, higher degree of polymerization and higher crystallization index than plant cellulose. Today, drug delivery derived from porcine or bovine is popular due to their availability and shorter processing time. Thus, many studies were conducted to find an alternative in order to produced product using natural based resources. The BC was synthesized in mature coconut water medium and had later undergone a seven days fermentation process. Characterizations of BC were conducted in comparison with hard gelatin capsule (HGC) films using Fourier Transform Infrared (FTIR) Spectroscopy, Field Emission Scanning Electron Microscope (FESEM) and X-ray diffraction (XRD) methods. Results from FESEM and XRD showed that BC films have higher stiffness with higher mechanical strength and crystallinity index than HGC from cattle bones. In addition, both films possess similar chemical moiety as showed in FTIR result.

Keywords: Bacterial cellulose, Hard gelatine capsule, Drug delivery.

INTRODUCTION

Bacterial cellulose (BC) was synthesized via fermentation to produce crystalline nanopolymer by gram-negative *acetobacter xylinum* 0416 bacteria. BC exhibit high degrees of polymerization, purity, tensile strength, water holding capacity, and crystallization index compared to plant cellulose [3]. It is generally used as a raw material in various industries such as paper, board, food, and textile [14]. Recently, BC has attracted the attention of one of the most important industries in the world which are medical and pharmaceutical industry. It is generally being used in skin therapy, artificial blood vessel, wound care products, and tissue engineering [13].

Gelatine is a biopolymer that is produced via hydrolysis of animal tissues. It is often used as a drug carrier, tissue scaffolds, and wound dressings [8]. However, the application of gelatine is limited because it has weak mechanical properties (as film) and highly soluble in water [8]. Over 50 % of food productions used gelatine and it is predicted to increase to approximately one hundred metric tons per year. However, the production of gelatine from porcine sources is almost 41 % compared to bovine skins (28.5 %) and bones (29.5 %) [12]. Porcine sources are preferred because it cost effective and shorter processing time. Moreover, gelatine from porcine sources only requires a month to produce compared to bovine sources, which could take approximately three months [9]. In 2016, the global market for gelatine was RM 6.12 billion and the forecast was predicted to increase by RM 9 billion in 2021 [12].

Drug delivery using natural polymers is preferable because they are readily available, inexpensive, low toxicity, biocompatible and potentially degradable [5, 8]. Nowadays, the use of various materials as a vehicle of medicaments is rapidly increasing, especially for coatings on medications or as packing agents. In the last decade, several biopolymers based such as polysaccharides have been developed to fulfil such demands [8, 10]. HGC is the gelatine capsule that usually found mostly in pharmaceutical industry and it is easily manufacture compared to other types of drug containers. This study was conducted to analyze BC properties for replacing the gelatine in HGC. BC and HGC films were characterized using FTIR, XRD, and FESEM.

MATERIALS AND METHODS

Bacterial strain

The microorganism used in this study was *acetobacter xylinum* 0416 and the bacterial stock culture was obtained from Malaysian Agricultural Research and Development Institute (MARDI).

Inoculum preparation

The inoculum mediumof *acetobacter xylinum* 0416 was prepared by dissolving 8 g glucose ($C_6H_{12}O_6$) and 0.5 g ammonium sulfate, $(NH_4)_2SO_4$ in 100 mL mature coconut water. The pH was carefully adjusted to pH 4.5 using 0.5 M

acetic acid (CH_3COOH). Then, the medium was sterilized using autoclave at 121 °C for 15 minutes. About 10 mL of stock culture was added aseptically into prepared medium. Then, the mixture was incubated for 3 days at 30 °C in incubator at static condition. After 3 days, the mixture was stored in a cold room at 4-8 °C.

Subculture preparation

GYC agar was used as a growth medium for acetoacter xylinum 0416. The GYC agar comprised of 2.5 g dextrose ($C_6H_{12}O_6$), 0.25 g calcium carbonate ($CaCO_3$), 0.5 g yeast extract, and 1.0 g agar was dissolved in 50 mL distilled water. The agar mixture was sterilized at 121 °C for 20 minutes. Then, the agar was poured into a petri dish and left to cool at room temperature before it was placed in a cold room overnight at 4 °C. A sterile inoculating loop was used to streak *acetobacter xylinum* 0416 onto the agar plate. The streaked plate was placed inverted and placed in the incubator at 30 °C for 3 days for bacterial growth [16].

Fermentation and recovery

About 10 mL of *acetobacter xylinum* 0416 inoculum was added into 100 mL of mature coconut water in a 250 mL conical flask. The pH was then adjusted to 4, 5 or 6 with 0.5 M CH_3COOH were incubated for 7 days at 30 °C in a static condition. After 7 days of fermentation, white pellicle of BC was observed on the surface of medium [1]. BC was then filtered from the conical flask, washed thoroughly and boiled in 0.5 M NaOH solution at 100 °C for 20 minutes to remove the bacterial cells [4]. Afterwards, BC was soaked in water and shaken vigorously until the colour returned to its original colour.

Dry weight of BC

The wet BC was placed in an oven at 60 °C until the weight became constant. The weight of the dried BC was measured using a weighing balance and the value was recorded and analyzed. Figure 1 showed overall BC production flow.

Figure 1: BC production

Preparation of HGC

The HGC derived from cattle bones was purchased from a pharmacy and was cut into specific measurements for characterization purpose. It was placed in an incubator at 15-25 °C with relative humidity of 35-65.

Characterization of BC and HGC

Dried BC film shown in Figure 2 was characterized together with HGC film. The properties of BC and HGC surfaces were observed using a high resolution Field Emission Scanning Electron Microscopy (FESEM) from MERLIN COMPACT. FESEM was operated at 3 kV with 10,000 times magnifications to examine BC and HGC morphology structure.

Figure 2: Dried BC film

A Fourier Transform Infrared (FTIR) Spectroscopy was used to identify and analyze the chemical structures of BC and HGC. The FTIR spectrophotometer used in this study was a Nicolet 6700 from Thermo Scientific, United States. The wavelength range used in this research was 1000 and 3600 cm^{-1}.

The crystallinity of BC and HGC were observed using X-ray diffractometer (XRD). This is due to the fact that XRD is sensitive towards crystallite size inside the particles. The XRD model was D8 Advance Bruker AXS from Germany at 40 kV and 40 mA of operating voltage and current, respectively. Both BC and HGC samples were scanned in a 10-30° range of 2Θ with 1.5406 nm wavelength. The samples were prepared by drying the solid samples, and they were cut into specific measurements before being mounted onto the smear slides. The samples were then placed into an X-Ray holder for analysis purposes. Equation 1 shows the cellulose crystallinity (%) calculation using following equation [15]:

$$\text{Cellulose crystallinity, \%: } (I_{002}-I_{am})/I_{am} \times 100\ \% \quad (1)$$

where, I_{002} and I_{am} are the maximum scattering intensities of the diffraction from the (002) plane at 2Θ = 22.6 and the background scatter diffraction intensity measured at 2Θ = 18°, respectively. Equation 2 shows the Scherrer formula used for crystallization size calculation:

$$\text{Crystallization size, L: } K\lambda/(\beta\cos\Theta) \quad (2)$$

where, K is the dimensionless crystallite shape factor (0.9), λ is the X-ray wavelength (nm), β is the line broadening at half maximum intensity (radians), and Θ is the Bragg angle (degree).

RESULTS AND DISCUSSION

Morphology

To ferment mature coconut water with *acetobacter xylinum* 0416, most researchers used static method in order to get a layer of thick gelatinous membrane. Mostly, *acetobacter xylinum* strain was used in the food industry to produce

dessert named as nata de coco [12]. The surface of BC and HGC films at 10,000x magnifications are shown in Figure 3. Figure 3 (a) showed the surface of BC film where the ribbons of cellulose fibrils were cross-linked together. This is because the BC pellicle was formed through micro fibrils bunch that was excreted from the pores of *acetobacter xylinum* 0416 and later aligned on the medium surface along the longitudinal axis. These ribbons of cellulose were tightly packed and stabilized by hydrogen bonding that exhibited stiffness and improved the mechanical strength of the BC film.

The observations on Figure 3 (a) of micro fibrils were similar to previous reported by Czaja (2004) while their morphological feature was similar with pure microcrystalline cellulose. Figure 3 (b) shows that the HGC film consisted a soft material that could not withstand harsh conditions due to the absence of the cross-linking images.

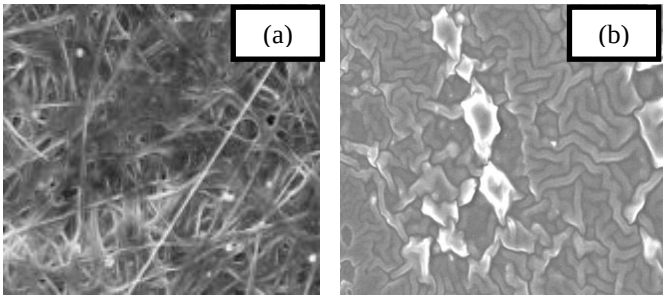

Figure 3: FESEM images for: (a) BC and (b) HGC at 10,000 X magnification.

The structure of cellulose powder from Sigma (standard) was compared with previous study from Halib et al. (2012). The results showed that cellulose powder (Figure 4) has different sizes, shapes and fibrous as compared to BC film.

FTIR Analysis

Figure 5 shows the FTIR spectrum of BC and HGC. The FTIR spectrum of BC film showed wavelength peaks at of 1577.6 cm^{-1} (carbonyl group), 1633.7 cm^{-1} (carboxyl group), 3354.0 1059.4 cm^{-1} (O-H stretching), and 1161.4 cm^{-1} (C-O-C functionality). These functional groups validated the chemical structure of BC ($(C_6H_{10}O_5)_n$, as shown in Figure 7. The FTIR results from this study were compared with previous findings from Surma-Sluraska et al. (2008), Gayathry & Gopalaswamy (2014), and standard cellulose from Halib et. al (2012). The result from this study indicated BC consisted of 1490 cm^{-1} (carbonyl group), 3400 cm^{-1} (hydroxyl group), and 1162 cm^{-1} (C-O-C functionality). The result was supported by the finding from Gayathry & Gopalaswamy (2014), which the FTIR conveyed the wavelength of 1644 cm^{-1} (carboxyl group), 1428 cm^{-1} (carbonyl group), and 1163 cm^{-1} and 1068 cm^{-1} (C-O-C functionality). The standard cellulose peaks at 1035-1060 cm^{-1} (carbonyl), 3350 cm-1 (hydroxyl), 1374 cm^{-1} (C-H stretching), and 1160 cm^{-1} (C-O-C functionality) has the same chemical group with FTIR results of BC in this study.

The spectrum for HGC film showed peaks at 1586.3 cm^{-1} (carbonyl group), 1652.2 cm^{-1} (carboxyl group), 3308.5 and 1082.6 cm^{-1} (O-H stretching), 1338.0 cm^{-1} (C-H stretching vibration), and 1110.6 cm^{-1} (C-O-C functionality). This showed the chemical properties of BC film are the same as the chemical structure of HGC films. The summary of major infrared (IR) for BC and HGC was shown in Table 1.

The FTIR spectrum for the comparison of BC with BC powder was shown in Figure 6. The wavelength of BC powder showed peak of carbonyl at 1056.9 cm^{-1}, 3350 cm^{-1} (hydroxyl), 1374 cm^{-1} (C-H stretching), and 1160 cm^{-1} (C-O-C functionality).

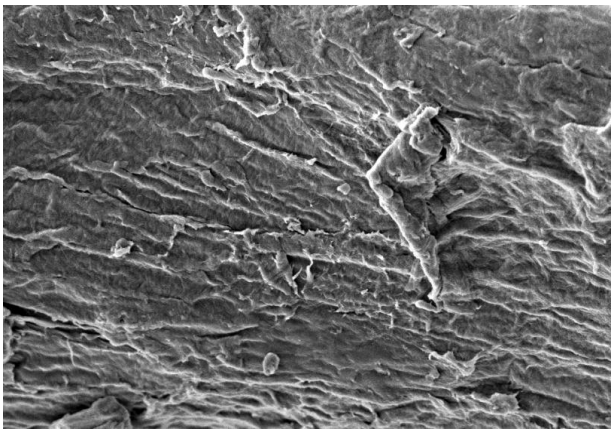

Figure 4: BC powder (standard)

Table 1: Major IR for BC and HGC

Types of film	Chemical moiety	BC				HGC (cm^{-1})
		Standard Cellulose (cm^{-1})	Research study (cm^{-1})	Surma-Sluraska *et al.* (cm^{-1})	Gayathry & Gopalaswamy (cm^{-1})	
Chemical group	Carbonyl	1438.6	1577.6	1490	1428	1586.3
	Hydroxyl	3350	3354.0 & 1059.4 (strong)	3400	-	3308.5 & 1082.6
	C-H stretching	1374	1338.0	-	-	1338.0
	C-O-C functionality	1160	1161.4 (strong)	1162	1163 & 1068	1110.6

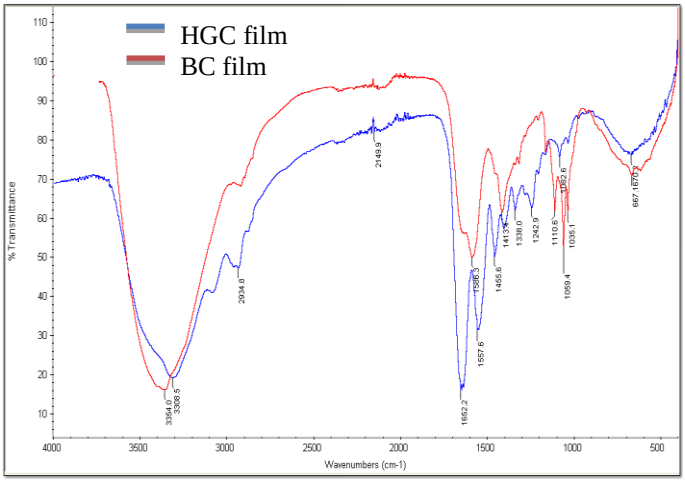

Figure 5: FTIR spectra for BC and HGC films

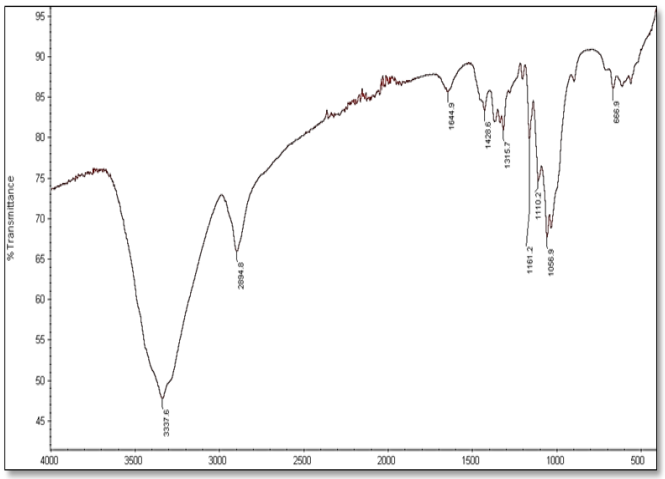

Figure 6: FTIR spectra for standard BC

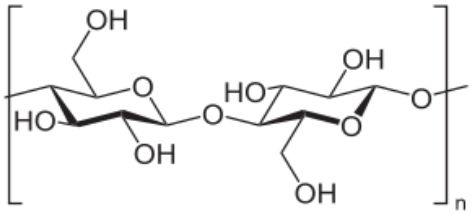

Figure 7: Chemical formula of BC [7]

XRD Analysis

Table 2: Percentage of crystallinity

Types of film	Crystallinity (%)
BC	99.41
HGC	9.7%

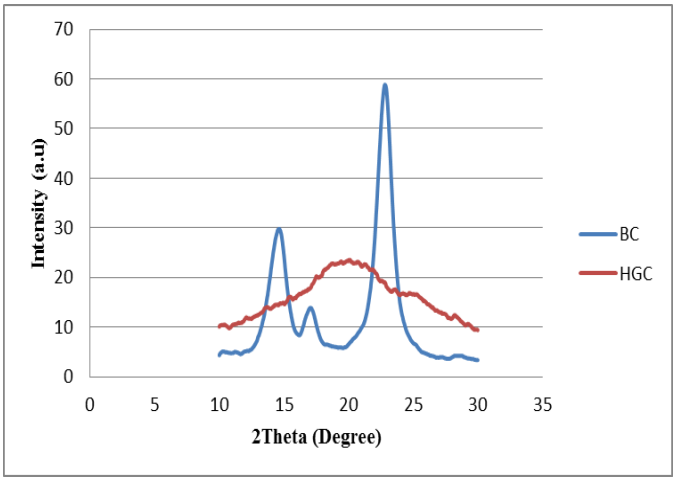

Figure 8: XRD patterns for BC and HGC

XRD was used to determine the crystallinity index and crystal size of both films. Figure 8 showed the XRD patterns of BC and HGC films. The main peaks for BC film were at 14.5, 17 and 23°. However, the main peak for HGC film was at 20°. The XRD analyses in Table 2 showed that BC has 99.41 % crystallinity index compared to 9.7 % for HGC. This result showed that BC has very high crystallinity index compared to HGC. According to Bhat et al. (2017), HGC is brittle due to the presence of water that acts as a plasticizer. Plasticizer is the substance that acts as reducing polymer stiffness because it can decrease the cohesive intermolecular forces along polymer chain [2]. XRD for pure cellulose was studied and showed the peaks at 14.5, 16.6 and 22.7 degree by Reddy et al. (2017). The diffraction peaks of pure cellulose were similar to BC film. Thus, it showed BC and pure cellulose has the same crystallinity percentage.

Table 3 showed the summary of both films that has been discussed in previous section. Even though both of them consisted of same chemical moiety, BC has more crystallinity and stability compared to HGC. The very low crystallinity percentage showed HGC is a soft material. Thus, due to its brittle disadvantage, the pharmaceutical manufacturers have faced the problem to manufacture and store the HGC. Therefore, the HGC manufacturer should replace from gelatine to BC to produce more elasticity product and develop more robust capsules.

Table 3: Summary of BC and HGC films

Types of Film	BC	HGC
FESEM (Morphology)	Tightly packed with cross fibrils (strong material)	Unpacked and does not have cross material (brittle material)
FTIR (Chemical moiety)	Carbonyl, carboxyl, hydroxyl, C-H stretching and C-O-C functionality.	Carbonyl, carboxyl, hydroxyl, C-H stretching and C-O-C functionality.
XRD (Porosity/crystallinity)	99.41% (more crystallinity)	9.7% (brittle)

CONCLUSION

The growing demand for halal food has subsequently led to high demand for halal gelatine. The demand for halal gelatine increases every year, thus, the study on the gelatine-substitute materials should be conducted in order to replace sources from pig and bovine. This study used BC film as a potential raw material to replace HGC. Characterization of BC and HGC were conducted and their chemical properties were observed. FESEM showed the ribbons of cellulose in BC. These ribbons were stabilized by the hydrogen bonds that gave the stiffness, high mechanical strength and high crystallinity to BC compared to HGC. In addition, HGC is also soft compared to BC due to the presence of plasticizer. This proven by XRD result which the percentage of BC higher than HGC. Meanwhile, the FTIR results showed that BC has approximately similar chemical properties as HGC. The XRD, FESEM and FTIR results of BC and HGC film were almost similar as compared to pure BC (standard). It shows that BC has highly potential to be as an alternative source of HGC.

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